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Greenhouse Gas Emissions Over a Tidal Cycle in a Freshwater Wetland

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Introduction

Tidal freshwater wetlands are located at the interface of non-tidal freshwater riverine systems and estuarine tidal systems. These habitats experience freshwater tides, creating unique redoximorphic soil characteristics while simultaneously presenting an opportunity for hydrologic nutrient transport into the system. Because of this periodic flooding and draining, tidal freshwater wetlands are systems of intense biogeochemical transformations, which are microbially mediated. Several microbial transformations (e.g., methanogenesis, incomplete denitrification, and nitrification) result in the production of greenhouse gases (CO₂, CH₄, and N₂O) at globally-significant levels. For example, wetlands are one of the greatest sources of methane on Earth, accounting for 20-33% of the global methane budget (Schlesinger and Bernhardt, 2013).

Compared to global methane emission estimates, the global nitrous oxide budget remains largely uncertain (Tian et al. 2015), and the contribution of wetlands is currently unknown (Schlesinger and Bernhardt, 2013). However, given that recent work by Liengaard et al. (2012) estimated that nitrous oxide emissions from the Pantanal wetland system in South America alone represent ~2% of global emissions, it is reasonable to expect wetlands to be major contributors to atmospheric concentrations of this potent greenhouse gas. Despite the growing recognition that wetlands are important sources of greenhouse gases, little research has examined how flux rates vary in response to basic environmental drivers such as tidal cycling

Objectives: The main objective of this study is to assess rates of CO₂, CH₄, and N₂O production at high and low tides in a tidal freshwater wetlands. In addition, we sought to determine if pore water ion concentrations and edaphic characteristics fluctuate over a tidal cycle.

Table 1. Dominant microbial pathways responsible for the breakdown of organic matter in wetlands (Reddy and DeLaune, 2008). Greenhouse gas end products are highlighted in blue.

Redox Potential	Electron Acceptor	End Products	Microbial Groups
Aerobic			
> 300 mV	O ₂	CO ₂ , H ₂ O	Aerobic heterotrophs
Facultative			
100 to 300 mV	NO ₃ ⁻	N ₂ O, N ₂ , CO ₂ , H ₂ O	Denitrifiers
100 to 300 mV	Mn ⁴⁺	Mn ²⁺ , CO ₂ , H ₂ O	Mn ⁴⁺ Reducers
100 to -100 mV	Fe ³⁺	Fe ²⁺ , CO ₂ , H ₂ O	Fe ³⁺ Reducers
Obligate			
< -100 mV	SO ₄ ⁴⁻	HS ⁻ , CO ₂ , H ₂ O	Sulfate Reducers
< -100 mV	CO ₂ , Acetate	CH ₄ , CO ₂ , H ₂	Methanogens

Methods

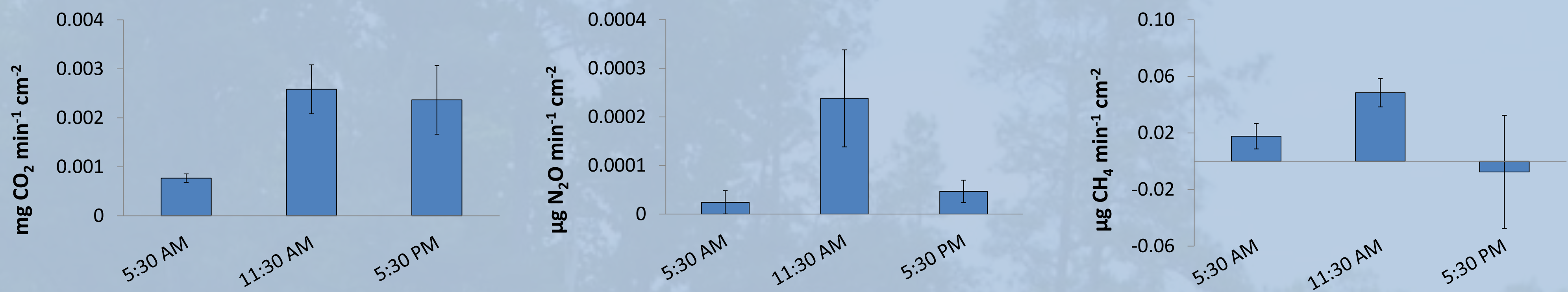
This goal of this study was to assess rates of greenhouse gas production, the abundance and expression of key microbial enzymes associated with these gases, and edaphic characteristics over a tidal cycle. The study was conducted on July 20th 2015 from 5:30 AM (low tide) to 5:30 PM (low tide), sampling at 2-hour intervals. A 5 x 5 m plot was establish in the tidal freshwater wetland at the VCU Rice Rivers Center in Charles City County, Virginia. Within the plot, walkways were established at 1-m intervals to prevent disturbance of soils during sampling. The sample plot was dominated by freshwater grass and forb species (*Leersia oryzoides*, *Murdannia keisak*, *Polygonum spp*).

Gas production: Air-tight chambers were constructed out of PVC (45.7 cm height x 15.2 cm diameter), with a total volume of 8.33 L. Chamber caps were equipped with a thermometer and a sampling port for collecting gas samples. For each sampling event, chamber caps were applied and gas samples (15 ml) were collect after 20, 40, and 60 minutes of incubation. At each of these times, chamber temperature, chamber pressure, and water level were recorded. Gas samples were later analyzed via gas chromatography.

Soil properties: For each sampling event (i.e., every 2 hours), 5 soil cores (5 cm deep x 10 cm diameter) were collected. Edaphic characteristics were measured using field probes for pH, redox, conductivity, and temperature. From the soil cores, ~2 g subsamples were collected and stored in MoBio LifeGuard solution for later genetic analysis. Cores were transported back to the laboratory on ice for extraction of pore water via centrifugation (3000 x g, 25 minutes) and filtration (0.22 µm) within 24 hours of collection. Pore water samples were later analyzed on an ion chromatograph.

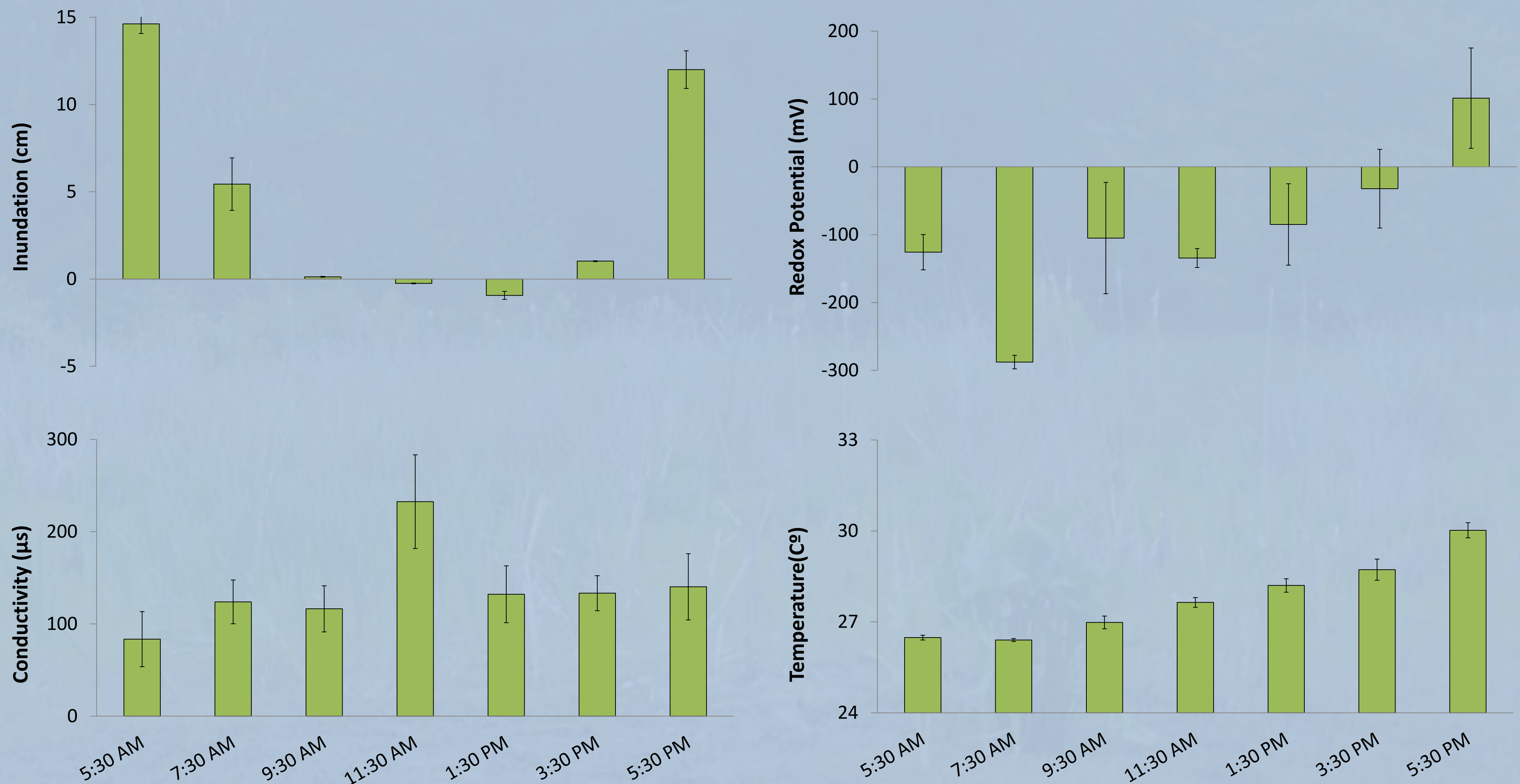


Greenhouse Gas Production



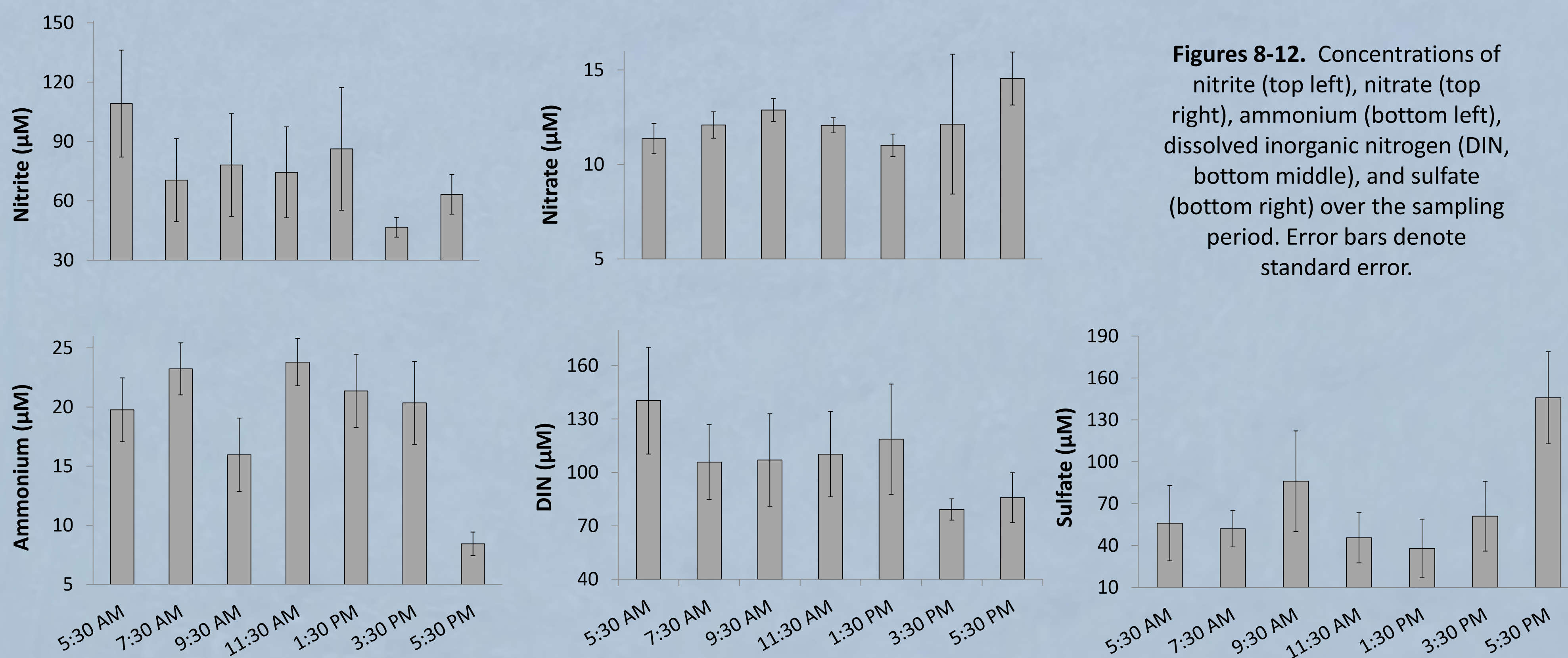
Figures 1-3. Production rates of carbon dioxide (left), nitrous oxide (middle), and methane (right) over the tidal cycle. Production rates are total production and include dissolved gases. Error bar denote standard error.

Soil Properties



Figures 4-7. Inundation recorded as water level relative to the soil surface (top left) and soil properties for 0-5 cm soil depth, including redox potential (top right), conductivity (bottom left), and temperature (bottom right). Error bars denote standard error.

Pore Water Chemistry



Figures 8-12. Concentrations of nitrite (top left), nitrate (top right), ammonium (bottom left), dissolved inorganic nitrogen (DIN, bottom middle), and sulfate (bottom right) over the sampling period. Error bars denote standard error.

Conclusions

- Soil redox** data suggest a lag between inundation and changes in soil redox potential, which is consistent with prior research (Ensign et al., 2008). Neither **pH** or **gravimetric water** content (data not shown) varied over the tidal cycle.
- Soil temperature** increased throughout the day, which may have favored increased CO₂ production at the 5:30 PM high tide. **Conductivity** showed a unimodal response over the tidal cycle.
- Average production rates for all **greenhouse gases** were the greatest at low tide (11:30 AM).
- While some **pore water ions** showed differences across time, no distinct tidal patterns were observed. Surprisingly, **nitrate**, which was hypothesized to increase due to the influx of river water during high tide, remained fairly unchanged over the sampling period.
- At the 5:30 PM high tide, pore water **sulfate concentrations** were the highest, coincident with decreased **methane production**. This could be because elevated sulfate stimulated organic matter breakdown by sulfate reduction rather than by methanogenesis, which is less thermodynamically favorable.
- Increased **methanotrophy** could also explain the decrease in methane production at 5:30 PM. Methanotrophs are a diverse set of prokaryotes capable of methane oxidation. Methanotrophs are facultative anaerobes, whereas methanogens are obligate anaerobes. Therefore, under anaerobic conditions both methanogenesis and methanotrophy can occur, while only methanotrophy can occur under aerobic conditions.
- It is possible that nitrification was occurring in the top layer of exposed soil during low tide. Therefore both nitrification and denitrification could both be contributing to elevated nitrous oxide production rates observed at this time.

Future Work

Whole-community RNA and DNA will be extracted from the archived soil samples; RNA will be reserve transcribed into cDNA for subsequent analyses. Quantitative PCR (qPCR) will be used to determine the abundance (DNA-based) and expression (cDNA-based) of key functional genes associated with microbial greenhouse gas production (Table 2). Genetics data will be correlated with gas production rates and edipathic characteristics.

Carbon dioxide, methane, and nitrous oxide production rates will be calculated for the four other sampling events. Due to high temporal variation in wetland ecosystems, this study should be repeated.

Table 2. Microbial functional groups to be analyzed via qPCR.

Functional Groups	Genes	Enzymes
Denitrifiers	<i>nirS</i>	Nitrite Reductase
	<i>nirK</i>	Nitrite Reductase
	<i>cnorB</i>	Nitric Oxide Reductase
Nitrifiers	<i>amoA-bacteria</i>	Ammonia Monooxygenase
	<i>amoA-archaea</i>	Ammonia Monooxygenase
Methanogens	<i>mcrA</i>	Methyl Coenzyme-M Reductase

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